Oxidation-Reduction Potentials of Canned Foods and Their Ability to Support *Clostridium Botulinum* Toxigenesis

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ABSTRACT

Oxidation-reduction potentials (Eh) of canned foods ranged from −18 to −438 mV. Foods packed in glass had higher redox potentials than those packed in cans. Only 4 out of 26 products tested reached positive redox values after exposure to air for 24 hr at 4°C. Inoculated containers of mushrooms, whole corn, cream corn, asparagus, beef gravy, kidney beans, green beans, cream of mushroom soup, cheddar cheese soup, and lima beans supported toxin production by *C. botulinum*; potatoes and beets did not.

INTRODUCTION

IT IS WELL RECOGNIZED that oxidation-reduction (redox) potential is an important growth determinant for both aerobic and anerobic microorganisms. Redox (Eh) values can range from +300 to −420 mV in different microbial cultures (Brown and Emberger, 1980). Barnes and Ingram (1956) observed that *Clostridium welchii* does not grow in pre-rigor meat due to its high (+250 mV) redox potential. *C. welchii* grows rapidly with no lag at −45 mV but has a very long lag at +216 mV before the onset of rapid growth. A redox value of +230 mV is completely inhibitory to *C. welchii*. Huss et al. (1980) have shown that the aerobic microflora associated with smoked fish can rapidly lower its Eh; toxin production by *C. botulinum* Type E is initiated at Eh levels as high at +100 to +250 mV. *C. botulinum* Type A and B toxin production has been reported in the Eh range of −6 to −436 mV (Odlaug and Pflug, 1978). The effect of oxygen and redox potential on *Clostridia* has been reviewed by Morris and O'Brien (1971).

Redox potential also interacts with other intrinsic factors to affect the ability of *Clostridia* to grow in a given system. Smoot and Pierson (1979) found that while *C. botulinum* exhibits comparable growth and toxin production in media at −60 and −145 mV, it is more salt sensitive at the higher redox potential. Redox mediated salt sensitivity also has been observed with *C. welchii* (Mead, 1969). Roberts (1970) has shown that the highest Eh permitting growth of *C. perfringens* is strongly influenced by pH.

In spite of its obvious significance, relatively little work on the redox potentials of foods has been published. While there are copious data on factors such as the pH, salt content, and nutrient composition of canned foods, to our knowledge, the only compilation of redox values lies in the PhD thesis of Merton Smith (1975). These values were based on single determinations for each product and remain unpublished. The dearth of information on this subject is due in part to the fact that redox potentials are notoriously difficult to measure reproducibly. Problems encountered include sample oxidation by atmospheric oxygen, differences in the poising capacity of different samples, side reactions in biologically active samples and in some cases, a long period required for the electrode to reach equilibrium with the sample and attain a reproducbiel value (Brown and Emberger, 1980).

As a prelude to our basic studies on the interaction of Eh with other variables to affect *C. botulinum* toxigenesis, we needed to determine what factors affect the redox potentials of canned foods. The objectives of this study were: to determine the redox potential of a variety of canned foods; to estimate the precision and accuracy of these measurements by obtaining multiple observations and comparing the redox potential of systems with published values (i.e., those obtained with laboratory media and redox indicator dyes); to determine whether the redox potentials obtained were an intrinsic property of the given horticultural product or varied with lot, manufacturer, and container type; and finally, to survey these products for their ability to support germination of *C. butulinum* spores and subsequent toxin production.

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MATERIALS & METHODS

Physiochemical methods

Triplicate samples of locally obtained commercially packed foods having the same lot number were analyzed as outlined below.

Oxidation-reduction potential and pH measurements were determined using a digital meter with slope control (Sargent Welch, Model PAX 9000). A flat surface membrane combination electrode (Fisher) standardized against buffers at pH 4.00 and 7.00 was used to measure pH. Redox measurements were made with a polished combination platinum redox electrode which contained a calomel reference electrode (Sargent Welch). This electrode was calibrated against the ferric-ferrous ammonium sulfate standard redox solution of Light (1972) which has an Eh of +470 mV.

The following procedure was used to determine the redox potential of the canned foods. The jar was opened under a tent of oxygen-free nitrogen. This tent was made by passing nitrogen through a copper filled furnace (Sargent Welch) to remove residual oxygen and then via Tygon tubing through an inverted Buchner (13 cm diam) funnel. The funnel was inserted through a small hole in the sealed end of a polypropylene bag (24 x 46 cm) and securely taped in place to form a cylindrical tent open at the bottom. The open end allowed exit of the nitrogen and insertion of the samples and electrode. The redox electrode was inserted into the lower half of the sample container, taking care not to contact the sides or bottom of the metal cans during the measurements. The redox potential in millivolts (mV) was read directly from the meter at appropriate intervals until the readings reached a plateau. This equilibrium value was recorded the the nearest mV. The Eh_7, the redox potential of the system standardized to pH 7 and 25°C, was calculated by algebraically rearranging the formula of Leistner and Mirna (1959) and substituting the correction factor 59 mv/pH at 25°C (Clark, 1960). The formula thus derived is:

$$Eh_7 = Eh \text{ measured} - 59(7.00 - pH \text{ measured})$$

An empirical index of the foods' ability to resist changes in redox potential (i.e., its poising capacity) was obtained by measuring the redox potential of each sample a second time after the opened container had been stored exposed to atmospheric oxygen at 4°C for 24 hr. The poising capacity was calculated as the percent change in redox potential between the first and second day readings.

The measurement of pH was made after the initial redox measurements by inserting the pH electrode just below the surface of the product.

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Table 1—Redox potentials, pH, and ability of canned foods to support toxigenesis by C. botulinum. The code consists of an arbitrary number and a letter assigned to each manufacturer. Data are presented in order of decreasing Eh_7 values

Product	Container	Code	Eh[a]	pH[a]	Eh_7[a]	Supports *C. botulinum* toxigenesis
Carrots, sliced	glass	5B	−018	5.06	−132	−[d]
Mushrooms, sliced	glass	11D avg	−137	6.01	−194	+[c]
		lot 1	−135	—	—	+
		lot 2	−132	—	—	+
		lot 3	−144	—	—	ND[b]
		99E	−135	5.95	−199	−
		10K	−137	5.91	−201	+
Beets, sliced	glass	99B avg	−102	5.12	−213	−
		lot 1	−95	—	—	−
		lot 2	−148	—	—	−
		lot 3	−64	—	—	ND
Blackeye peas	can	99F	−178	6.22	−225	ND
Beets, sliced	glass	1H	−140	5.18	−247	−
Green beans, cut	glass	17F	−149	5.12	−260	−
Onion soup	can	99A	−169	4.80	−299	ND
Asparagus	glass	20J	−225	5.46	−316	+
Corn, whole	can	99G avg	−305	6.57	−330	+
		lot 1	−335	—	—	+
		lot 2	−302	—	—	+
		lot 3	−277	—	—	ND
Beef gravy	can	49N	−301	5.61	−383	+
Cream corn	can	27H	−326	5.93	−389	+
Corn, whole	can	37B	−384	6.56	−410	+
Sauerkraut	can	26H	−203	3.43	−414	−
Cream of mushroom soup	can	29A	−375	6.24	−420	+
Spinach	can	99B	−318	5.19	−425	ND
Kidney beans	can	28M	−367	5.88	−433	+
Green beans, cut	can	34L	−361	5.62	−442	+
Tomato juice	can	99A	−293	4.30	−452	ND
Potatoes, whole	can	48A	−369	5.60	−452	−
Cheddar cheese soup	can	30A	−384	5.73	−459	+
Mushrooms, sliced	can	9I	−421	6.24	−466	+
Asparagus	can	21E	−374	5.40	−468	+
Lima beans	can	18B	−444	6.08	−498	+
Beets, sliced	can	2H	−409	5.45	−500	−
Mushrooms, sliced	can	8E	−445	5.92	−509	+
Beets, sliced	can	4B	−395	5.04	−510	−
Beets, sliced	can	3L	−410	5.20	−516	−
Beef stew	can	50B	−446	5.73	−521	+
Bartlett pears	can	99H	−339	3.88	−523	ND
Green beans, cut	can	99B-C avg	−416	5.20	−523	+
		lot 1	−443	—	—	+
		lot 2	−410	—	—	+
		lot 3	−396	—	—	ND
Applesauce	can	99P	−342	3.51	−547	ND
Tomatoes, whole	can	22C	−397	4.36	−553	−
Carrots, sliced	can	4B	−438	5.04	−554	±[e]

[a] Average of three determinations
[b] ND = Not done
[c] + = Toxic
[d] − = No toxin detected
[e] ± = Weakly toxic

Both redox and pH measurements of each product were made on triplicate containers bearing the same manufacturer's lot code number. In some cases multiple lots and lots of the same product produced by different manufacturers were analyzed to compare the constancy of the redox value for a given product.

In order to obtain redox potential values for comparison with published values, we also measured the redox potentials of some nonfood systems. These were the redox potentials of the sodium thioglycollate medium of Reed and Orr (1942) and of tryptic soy broth (Difco) as well as the redox potential at which 0.001% methylene blue (Sigma) became colorless in 0.1% peptone (Difco) adjusted to pH 7.0 when oxygen free nitrogen was bubbled through it.

Microbial methods

To determine the ability of the heat processed foods to support *C. botulinum* toxigenesis and to determine if redox potential was an important factor, post-process inoculation experiments were conducted. The septum method of Stersky and Thacker (1980) was used to inoculate each product with a suspension containing 10^5 heat shocked (80°C, 10 min) spores of *Clostridium botulinum*. The preparation of the mixed spore suspension which contained equal numbers of spores from strains 62A, ATCC 25763, B-aphis, and 53-B has been previously described (Montville, 1981). No heat treatment was applied to the inoculated cans. The cans were incubated at 35°C and observed for swells.

Every inoculated can was assayed for botulinal toxin after 60 days of incubation. A portion of the liquid from each can was centrifuged at 8,740 x *g* for 90 sec in a Microfuge B (Beckman). If the viscosity of the product was too high, it was diluted with an equal volume of gel phosphate buffer (Dowell and Hawkins, 1979) prior to centrifugation. The supernatant was used for the toxin assays. Each sample was tested for botulinal toxin by injecting each of two 15–20g Swiss white mice intraperitoneally with 0.4 ml of the putative toxin. Control mice were injected with samples that had been boiled for 10 min. The mice were observed for symptomatic botulinal death for 72 hr. All deaths attributed to botulinal toxin occurred within 24 hr.

RESULTS & DISCUSSION

TABLE 1 PRESENTS the redox data in order of descending Eh_7 values and summarizes the results of 139 individual redox determinations. The redox potentials of the foods studied were universally low, ranging from Eh −18 mV to −446 mV. Because all of the foods studied were at least weakly acidic, the recalculation of the data to Eh_7 made values even lower. There is some disagreement about the wisdom of correcting Eh values to pH 7. The factor of 59 mV per pH unit is derived from known redox couples involving one dissociating hydrogen. It is not valid for couples which are not hydrogen mediated or which have multiple ionizable groups (Hewitt, 1950). Since the stoichiometry of the redox couples in the foods studied is unknown, the validity of the Eh_7 standardization is not certain. Eh_7 values have been calculated and included because they are widely used; the original Eh values are also included. It should be noted that the use of Eh_7 rather than Eh has no qualitative effect on the interpretation of the data.

One of the most easily noted features of the data is that the redox potentials for products packaged in glass containers were generally higher than the Eh of products packaged in cans. With the exception of blackeye peas, onion soup, and whole corn, all of the redox values more positive than Eh_7 −350 mV were obtained from foods packed in glass containers. In the case of the same product packed in both container types, the glass-packed product always had a higher redox value. Carrots packaged in glass by manufacturer B had an Eh_7 of −132 mV, the highest value recorded. The lowest Eh_7 obtained, −554 mV, was from the same manufacturer's carrots packed in cans. Similar results were obtained with mushrooms, beets, green beans, and asparagus. This is somewhat surprising since foods are packed in glass under a higher vacuum than those packed in cans. Comparisons of additional products packed both in

glass and in cans could not be made because there are relatively few such products on the local retail market. The reason for the effect of container type on redox potential is not obvious. One possibility is that the higher Eh values of the glass-packed products were due to photo-oxidation of the product.

The redox potentials of a given product packed by different manufacturers had similar values. Mushrooms packed in glass had redox potentials ranging from Eh_7 −194 to −201 mV; packed in cans they ranged from −466 to −509 mV. Similarly, beets packed in cans had potentials ranging from −500 to −516 mV; in glass they were −213 and −247 mV. This suggests that the redox potential is an intrinsic property of products packed in the same type of container, but again calls attention to the substantial container effect.

The triplicate Eh determinations made for different samples within each product lot were within a 50 mV range (data not shown). No marked differences between lots were observed with the products tested (Table 1). Single, spot check determinations were repeated for several products. These results were always within 50 mV of the values shown in Table 1.

The Eh_7 values for sliced beets, lima beans, cut asparagus, mushrooms, sauerkraut, green beans, and blackeye peas obtained in this study were comparable to (within 25%), but generally lower than those reported by Smith (1975). Some products (cheddar cheese soup, cream of mushroom soup, cream corn, peas, Bartlett pears, and spinach) however, had redox values more than 200 mV more negative than those of Smith. The origin of these differences is unclear. It is possible that the foods had not reached equilibrium with the electrode when the higher values were obtained. The period required to reach equilibrium can be quite long (Fig. 1). The difference between the equilibration curves for applesauce and blackeyed peas demonstrates that the equilibration time depends on the product and is similar but not identical for different samples of the same food. Some foods such as applesauce (Fig. 1a) reach equilibrium fairly rapidly. Other products (Fig. 1b) are electromotively sluggish and take an exceedingly long time to reach equilibrium. Most foods tested were between these two extremes, (Fig. 1c). It should be noted, however, that in all three cases, values for triplicate samples were within 25 mV, once equilibrium was reached.

Most of the products studied were extremely well poised (Table 2). In this table, because all of the initial redox values of the samples were negative and increased with time when exposed to atmospheric oxygen, a change of greater than 100% was required to reach aerobic conditions (as evidenced by positive redox potentials). Only four out of twenty-six products thus measured become aerobic in this sense. While these measurements were made in the lower half of the container and did not reflect the redox gradient established in the can, they did show that some products wee remarkably able to resist changes in their redox potential. Indeed, the changes observed in the eight most poised products were in the range of experimental error. This indicates that exposure to air, in and of itself, did not necessarily make a food aerobic. The redox potentials of carrots, green beans, and asparagus were much higher on the second reading for the glass-packed products (which had higher initial redox potentials), but the redox potentials did not change much for the products in cans (which had lower initial values) when they were

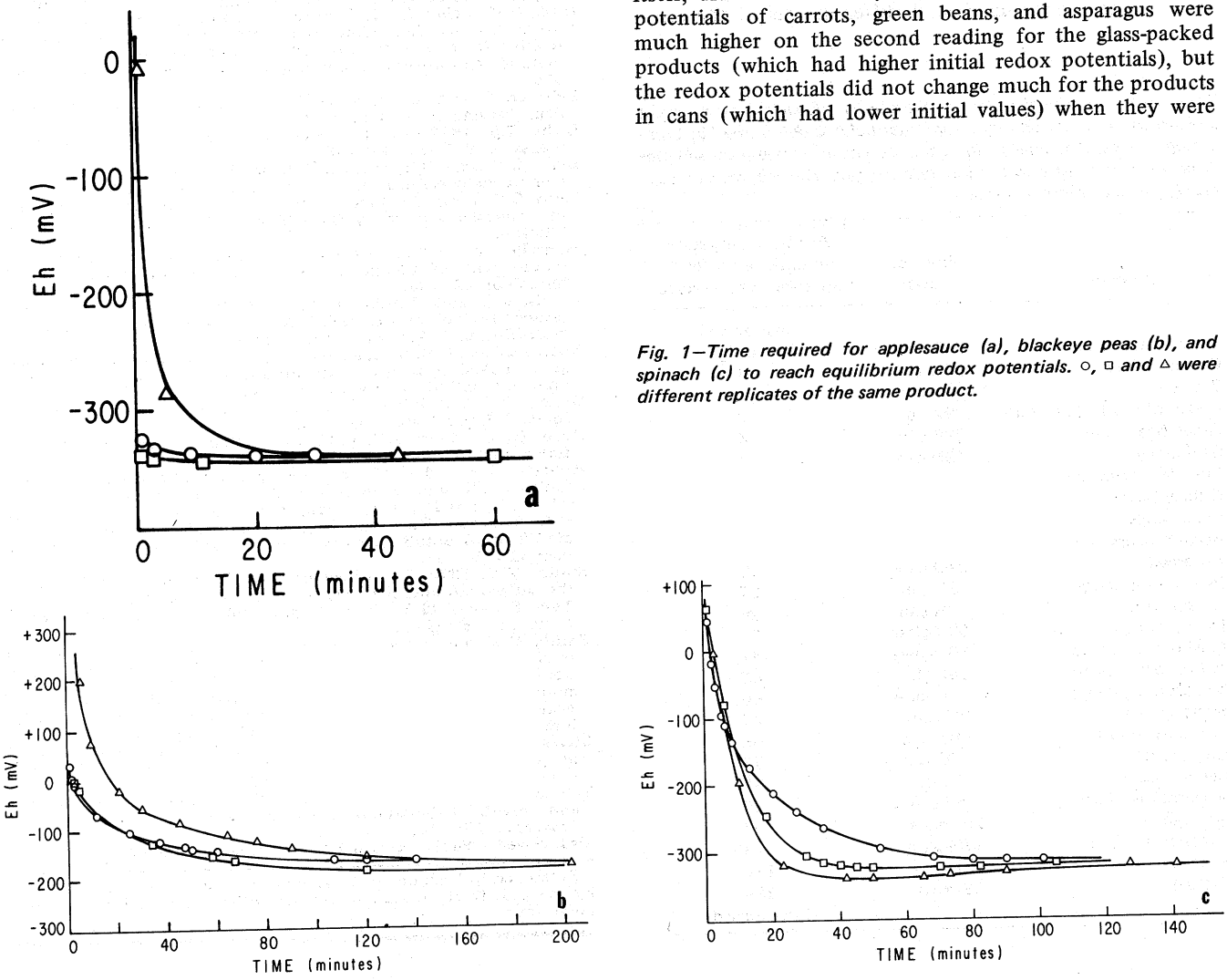

Fig. 1—Time required for applesauce (a), blackeye peas (b), and spinach (c) to reach equilibrium redox potentials. ○, □ and △ were different replicates of the same product.

exposed to air. One might have expected the opposite; that the most reduced products would exhibit the greatest increase in redox potential when exposed to air because of a greater driving force generated by their very negative initial redox potentials. It is possible that the poising capacity of the glass-packed products was almost exceeded during storage, thereby allowing rapid oxidation of these products when they were exposed to air.

The accuracy of the redox measurements of the canned foods in this study was supported by comparison of the redox potentials of nonfood systems with published values. The Eh of sodium thioglycollate medium in this study was −227 mV, slightly lower than the values of −175 to −200 mV reported by Reed and Orr (1942). The published values for tryptic soy broth are +40 mV (Tabatabai and Walker, 1970) to −140 mV (Smith and Pierson, 1979). The redox value determined in this study was −102 mV. The value at which methylene blue becomes colorless is −49 mV (Clark, 1960). When measured under conditions used in this study, a value of −6 mV was obtained.

The majority of the foods tested were able to support toxin production by *C. botulinum* (Table 1). While these experiments were not designed to demonstrate cause and effect, it is interesting to note that green beans, carrots, and mushrooms (mfg E), which were packed in glass and had higher redox potentials, were unable to support toxigenesis while the same products which had been packed in cans and had lower redox potentials did become toxigenic when inoculated with spores and incubated.

Given the notoriety of mushrooms as a food with botulinic potential (Lynt et al., 1975), our inability to detect toxin in any jar from several different lots of the glass-packed mushrooms of manufacturer E was perplexing. Mushrooms packed in cans by the same manufacturer (E) did support toxigenesis, as did all of the mushrooms packed by other manufacturers.

It is also interesting to note that, while cans of inoculated potatoes did swell on incubation, no toxin was detected. Potatoes have been reported to be an ideal substrate for growth and toxin production by *C. botulinum* (Seals et al., 1981; Notermans et al., 1981).

In summary, canned foods had low redox potentials. The redox potentials appeared to be an intrinsic property of the food, but were highly influenced by whether the foods were packed in cans or in glass jars. The foods were well poised, but because many were electromotively sluggish, the redox potentials were difficult to measure. The redox potential of certain foods may influence their ability to support toxin production by *C. botulinum*.

Table 2—An empirical index of the ability of each food to resist changes in its oxidation-reduction potential was determined by comparing the percent change in redox potential of triplicate samples after 24 hr exposure to atmospheric oxygen. The laboratory codes are the same as those in Table 1

Product	Laboratory code	Avg % inc. in redox potential after 24 hr exposure to atm oxygen
		most poised
Tomatoes, whole	99P-can	0.5
Carrots, sliced	6B-can	1
Beef stew	50B-can	1
Cream of mushroom soup	29A-can	3
Green beans, cut	99B-can	4
Beef gravy	49N-can	4
Cheddar cheese soup	30A-can	6
Kidney beans	28M-can	11
Lima beans	18H-can	13
Bartlett pears	99H-can	15
Asparagus	21D-can	16
Mushrooms, sliced	8E-can	27
Cream corn	27H-can	28
Beets, sliced	99B-glass	37
Mushrooms, sliced	10K-glass	41
Mushrooms, sliced	14E-glass	42
Mushrooms, sliced	11D-glass	46
Beets, sliced	2H-can	47
Potatoes, whole	48H-can	65
Corn, whole	15G-can	75
Beets, sliced	3L-can	83
Mushrooms, sliced	9I-can	99
Asparagus	20J-glass	103
Green beans, cut	17H-glass	113
Sauerkraut	26H-can	117
Carrots	5B-glass	389
		least poised

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